HEMODYNAMIC AND METABOLIC ASSESSMENT OF ISOLATED CANINE HEARTS SUSTAINED BY A MODIFIED PERFUSION OF NONHOMOLOGOUS BLOOD

STEVEN BRENT SLOVIC

1980

HEMODYNAMIC AND METABOLIC ASSESSMENT OF ISOLATED CANINE HEARTS FOLLOWING TWENTY-FOUR HOUR PRESERVATION IN INTRACELLULAR-TYPE FLUID

Steven Brent Slovic

B.S., M.S. Stanford University 1984

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Medicine

Yale University School of Medicine

1988

Faculty Advisor: Dr. John A. Elefteriades, Department of Cardiothoracic Surgery

Acknowledgements

Thank you!:

Dr. John Elefteriades, my faculty advisor, for taking the time to assist me with this thesis project

Spyros Condos, James Keane, and Marie Longo for excellent technical assistance in the surgical laboratory

Dr. Graemme Hammond for thoughtfully reviewing this work and offering suggestions while it was in progress

Gail Thomas for administrative assistance and for helping me keep "in touch" with Dr. Elefteriades

Mom and Dad!--for all of your help and support during these past 8 years of college and medical school

TABLE OF CONTENTS

I. ABSTRACT

HEMODYNAMIC AND METABOLIC ASSESSMENT OF ISOLATED CANINE HEARTS FOLLOWING TWENTY-FOUR HOUR PRESERVATION IN INTRACELLULAR-TYPE FLUID

Steven Brent Slovic

1988

I. ABSTRACT

A Langendorf preparation with a left ventricular balloon was used to evaluate the isovolumetric function of six dog hearts before and after twenty-four hour preservation in a four-degree Celsius bath of intracellular-type fluid(ICF). Each heart served as its own control and cross-perfusion from a support dog was used during hemodynamic assessment of the isolated hearts. Mean left ventricular pressure development (LVPD), left ventricular positive dp/dt (LV dp/dt) and diastolic compliance were measured at baseline (pre-storage) and again 0.5, 1.0, 2.0, and 3.0 hours following twenty-four hour preservation and blood reperfusion. At each time point oxygen and lactate content were measured in samples of arterial and coronary sinus blood. Gravimetric water content was determined prior to storage and following three hours of reperfusion.

All six hearts beat upon blood reperfusion and showed preserved contractile and metabolic function. The average LVPD and LV dp/dt reached baseline after two hours of reperfusion. The diastolic compliance after preservation (0.50 ± 0.26 mmHg), was significantly decreased from baseline (1.90 ± 0.26 mmHg), but did not change during the three-hour

period of blood reperfusion. The water content of the left ventricular tissue increased from 78.7 ± 0.3 ml/100gm for freshly excised hearts to 82.1 ± 0.4 ml/100gm after twenty-four hour storage and 3 hours of blood reperfusion ($P<0.05$). Oxygen consumption and lactate extraction after preservation were not significantly different from baseline values.

We conclude that: 1) Twenty-four hour preservation by the simple technique of immersion in cold, intracellular-type fluid effectively preserves hemodynamic function as manifested in both pressure development (LVPD) and "contractility" (LV dp/dt); 2) Hemodynamic function is depressed initially after twenty-four hour preservation and subsequently improves progressively with blood reperfusion, reaching pre-preservation levels after three hours; 3) The recovery of hemodynamic function occurs despite decreased diastolic compliance and the presence of myocardial edema; and 4) Recovery of hemodynamic function corresponds with the return of metabolic function towards baseline.

II. INTRODUCTION

II. INTRODUCTION

A. The Shortage of Donor Hearts

This past December 2nd marked the twentieth anniversary of the first human heart transplant by Dr. Christiaan Bernard in South Africa. Today heart transplantation is a real therapeutic technique for patients with end-stage heart disease partly due to the introduction of cyclosporine in 1980 to combat rejection; In 1986 more than 2000 heart transplants were performed worldwide and it is predicted that about 4000 will have been performed in 1987(64). The most experienced centers report actuarial survival rates between 80% and 100% at one year and 50% at five years. These rates compare favorably to 42% one-year survival for patients in need of organs but denied eligibility. Furthermore, 5% of Stanford's heart transplant recipients who have lived one year have been able to return to normal occupation and activity (67).

Despite such clinical success and the improvements in treating rejection, cardiac transplantation is still faced by a major barrier--the ever present lack of donor organs! An estimated 14,000 people could benefit from a heart transplant every year in the United States (20) and at least 1000 of them (approximately one of four patients actually on a

waiting list) may die before a donor heart becomes available (13,83). Thanks in part to media coverage, the public awareness of the need for donor organs has increased. Nonetheless, the supply of donor hearts is likely to be even further reduced by the introduction of seatbelt laws, laws requiring the use of child-restraint seats, handgun laws, and motorcycle helmet laws (21). The fear of transplanting organs infected by Human Immunodeficiency Virus (HIV) is also likely to eliminate more potential donor organs from consideration in the coming years.

Several years ago the use of cardiac xenografts (hearts taken from another animal species) promised to provide easy availability of donors. However, graft survival in animal testing has been poor and xenografting cannot be considered a viable alternative until the major problem of histoincompatibility is overcome (52,84). The questions of public acceptability and the possibility that animal organs may harbor infectious agents that may be transmitted to the human recipient must also be considered.

The advent of the Jarvik artificial heart in 1982 also failed to eliminate the shortage of donor hearts and introduced other ethical and technical problems. Cardiac support by a mechanical device is currently a viable option for individuals whose clinical condition deteriorates while awaiting a human heart. However, as long as the shortage of transplantable human hearts exists, the use of temporary artificial hearts as "bridges" to a human heart cannot

increase the total number of human lives saved by heart transplants (1). Patients with artifical devices are given higher priority than patients being medically managed while awaiting a donor heart, thereby only changing the identity of the people who obtain the available human hearts.

Since cardiac transplantation is not likely to be superseded soon by xenografting or artificial devices, the donor-shortage problem will need to be resolved if it is to develop fully as a therapeutic clinical procedure. Extending the effective period of storage of hearts will be one way to increase the donor pool; Long-term (twenty-four hour) storage will allow truely remote harvesting--essentially from all regions of the world-- enabling the use of organs that would otherwise be "wasted".

A recent retrospective review of 233 potential cardiac donors referred over six years to the University of Arizona Health Sciences Center showed that the largest category (31%) of all donor refusals consisted of 50 patients, otherwise acceptable, who were refused as cardiac donors because an ABO-compatible recipient was not readily available (18). This suggests that long-term storage will not only allow time for more in-depth histocompatability matching, but would also effectively expand the donor pool by providing time to distribute organs to ABO-compatible recipients.

The current method of heart procurement in most active transplant centers utilizes storage of the donor hearts in a bucket filled with sterile saline at four degrees Celsius.

Experimental and clinical studies have shown that good protection achieved in this manner is limited to four to six hours (4,8,14,57). The present study evaluates the use of an intracellular-type fluid that mimicks the normal ion composition of fluid in cells for long-term myocardial preservation. Its design was based on established principles of myocardial protection and the experiences of other investigators in the field of organ preservation.

B. Principles of Myocardial Protection

A heart may suffer ischemic damage during harvesting from the donor or during the operation to transplant it into the recipient. However, Burt and Copeland recently quantified the decrement in function of rabbit hearts at each step in the transplantation process and found that the greatest damage occurs during the period of cold storage(11). The challenge to protect the heart during this time of cold ischemia is similar to the problem encountered during cardiac surgical procedures such as coronary artery bypass grafting and valvular replacement. Ideally the solutions used for cardioplegic protection during clinical open-heart surgery or for organ storage should: 1) provide immediate pharmacologic arrest to lower energy demands and avoid energy depletion by ischemic electromechanical work; 2) use hypothermia to reduce energy demands further and prevent recurrence of electromechanical activity; 3) provide substrates for continued aerobic or anaerobic activity; and 4) prevent or reduce unfavorable interstitial and cellular changes such as edema(9).

Potassium has been the mainstay of almost all cardioplegic and organ-preservation solutions since solutions containing high potassium concentrations were first used in 1955 to facilitate cardiac surgery(45,55). Extracellular

hyperkalemia provides immediate cardiac arrest by depolarizing cell membranes and produces a state of sustained diastole. The blockade of contractile activity minimizes oxygen demand and eliminates ATP-expending actin-myosin interaction. The safe upper limit of potassium concentration remains unclear. However, Tyers et al. found that functional deterioration equivalent to that seen with sixty minutes of normothermic ischemic arrest resulted only when hyperkalemia twenty-five times in excess of that required to arrest the heart (about 10 mEq/L) was used(76).

Hypothermia is also used in organ protection to reduce energy requirements and oxygen demand. The question of the optimal temperature to achieve maximal protection cannot be unequivocally answered from the available data. Angell and Griepp and their colleagues demonstrated in two separate studies with canine hearts that the duration of allowable ischemia is a linear function of the logarithm of temperature(2,31). These studies and another by Tyers et al. using isolated rat hearts (75) found that saline or Krebs-Henseleit (extracellular-ion composition) buffers at ten to fifteen degrees Celsius gave better functional and metabolic preservation for relatively short-term(one to four hour) cardiac ischemia than four degree or twenty degree Celsius solutions. In contrast, Swanson et al. observed that canine hearts preserved at four degrees Celsius for five hours functioned at levels equal to or greater than hearts stored at fifteen degrees Celsium and they found no evidence of

myocardial damage attributable to the cooler temperature alone(71).

The studies mentioned above were in agreement that temperatures approaching zero degrees, even for short periods, had a deleterious effect. Although red blood cells and bone marrow are successfully preserved by freezing, only limited success has been achieved using cryopreservation of hearts. Offerijns and Krijnen found that good preservation of rat hearts in a supercooled state (-18 degrees Celsius) for longer periods than one or two hours was impossible even though dimethyl sulfoxide (DMSO) was employed as a cryoprotectant(60). Likewise, Bausamian et al. found that freezing of puppy hearts at -2 degrees Celsius produced irreversible injury precluding resuscitation(3). However, using a combination of mechanical dehydration and glycerol saturation, the same group successfully transplanted several puppy hearts that had been cooled to -8 degrees Celsius for several hours without freezing. Therefore, these investigators concluded that it is the insult of freezing and thawing, not cooling per se, that irreversibly damages hearts.

The relative importance of hypothermia and potassium-based chemical cardioplegia in myocardial protection has also been studied. It appears clear that they have separate and additive properties(34,72).

Details of the molecular mechanisms involved in tissue injury are presently unknown. Purported mechanisms include

depletion of the cellular high-energy phosphate supply, accumulation of toxic waste products, and alteration of transmembrane ionic gradients with resultant cellular swelling (37). Effective hypothermia and cardioplegic arrest appear to preserve cytosolic adenosine triphosphate (ATP) levels well (25,42). It has also been well established that the cellular swelling that characterizes both ischemia and hypothermia can be greatly reduced by making preservative solutions hyperosmolar by the addition of solutes such as glucose or mannitol (27,28,49). Other compounds, including steroids and phenothiazines as "membrane-stabilizers", have been used in attempts to aid myocardial protection. In fact, so many agents were being tested that Katz was prompted to calculate that about a million experiments could be done to characterize the interactions and effects of just thirty cardioplegic solutions(39)! In the discussion that follows, some of the most promising and more recent approaches to the problem of long-term cardiac preservation will be reviewed.

C. Techniques of Long-Term Preservation

The first successful attempt to maintain hearts outside of the body for more than a few hours is attributed to Lindbergh, who in 1935 used a self-contained, pulsatile perfusion apparatus(51). *In vitro* perfusion has remained a popular technique for long-term preservation of the heart.

In 1969 Feemster et al. used hyperbaric, pulsatile perfusion at twenty-four degrees Celsius (with a continuously fibrillating heart) to attempt long-term cardiac preservation (24). Many other workers have used continuous, low-pressure, hypothermic (four to ten degrees Celsius) perfusion with more success. This technique is thought to provide a continual supply of oxygen and substrates as well as removal of waste products. Proctor and Parker introduced low-pressure perfusion in 1968 and preserved dog hearts for 72 hours with return of "strong contractions", as judged by gross observation, upon blood reperfusion(63). In the early 1970s, several groups of investigators demonstrated survival of animals following orthotopic transplantation with grafts preserved for 24 to 28 hours using this technique(17,62,74). Trunkey et al.(1970) used oxygenated, filtered canine plasma and mimicked contractions with external compression by a silastic balloon to perfuse hearts without edema. Eight hearts preserved in this manner for twenty-four hours initially functioned well after orthotopic transplantation,

but began to fail after four hours(74). Proctor et al.(1971) transplanted sixteen hearts following 72 hours of perfusion with Krebs crystalloid with survival in ten animals up to a maximum of fourteen hours(62). Copeland et al.(1973) also used hypothermic perfusion with a modified Krebs solution, along with pretreatment of the donor animal with phenoxybenzamine and glyceryl trinitrate. An overall surival rate of 30% was achieved at twenty-four hours following orthotopic transplantation of the preserved hearts (17).

Encouraged by these earlier reports, Guerraty et al.(1981) performed a similar study using canine hearts preserved twenty-four hours with continuous hypothermic perfusion with modified Krebs solution. However, the addition of post-operative immunosuppression to their protocol improved survival to 73% at one day following orthotopic transplantation and a 20% survival rate was achieved at thirty days post-op(32). In addition, Miller et al.(1984) stored hearts for twenty-four hours with continuous perfusion with oxygenated Krebs at five degrees Celsius and compared this method of preservation to simple-storage (non-perfused) in a five-degree bath of Ringer's lactate. Ventricular pressure development and positive dp/dt were measured following orthotopic transplantation. The ventricular function of the hearts preserved by perfusion was virtually identical to control, untransplanted hearts in open-chest dogs. The hearts stored in the cold bath of Ringer's lactate showed depressed function, 60% to 80% of

control values(57).

Wicomb and his colleagues in South Africa used this technique to perfuse baboon hearts for twenty-four hours and after orthotopic transplantation achieved consistent survival until rejection(16,81). Following this success in the experimental laboratory, Wicomb et al.(1984) reported the only clinical use to-date of hearts following long-term preservation. Four patients underwent heterotopic transplantation with donor hearts preserved from seven to seventeen hours by continuous perfusion with oxygenated, hyperosmolar, standard cardioplegia. Three of the four donor hearts required considerable support by the recipient circulation and inotropes for up to twenty-four hours post-op and in two cases the donor hearts would not have supported the circulation initially if orthotopic transplantation had been performed. All four hearts subsequently recovered normal function, although one failed shortly secondary to acute rejection(80).

Despite the attention that perfusion techniques have received, it is apparent that perfusion per se is not absolutely essential for successful preservation. The choice of storage solution also seems to play a significant role in determining the outcome of organ storage. As will be described shortly, intracellular-type fluids have been used very successfully both to perfuse isolated hearts continuously for twenty-four hours and for simple, non-perfused storage. Furthermore, orthotopic transplantation is

not the only way in which cardiac function has been assessed following preservation. Like the present study, Bethencourt(7) and Levitsky(50) used intraventricular balloons to study the function of isolated canine hearts perfused with cold, modified Krebs solution and canine plasma respectively. A comparison of ventricular function curves for the preserved hearts versus a group of control hearts demonstrated that good preservation was achieved.

In the future, several relatively new approaches may prove beneficial in prolonging the viable hypothermic ischemia time. Fluosol DA, a perfluorochemical with a very high oxygen-carrying capacity is being used experimentally as a myocardial protectant (19,35,38,43). Free radical scavengers, such as superoxide dismutase, seem to prevent the injury caused by oxygen free radical generation during blood reperfusion of stored organs(69). Constant-strength, high intensity magnetic fields have also been found to reduce mitochondrial injury in preserved hearts(29).

C. The Use of Intracellular-Type Fluid (ICF) for Organ Preservation

Theoretical value of ICF. Biopsies of ischemic myocardium show that hypothermia and standard cardioplegia are able to maintain high levels of cytosolic ATP. However, immediately upon reperfusion with warm blood and the onset of contractions, there is a rapid fall in ATP. ATP levels may reach or fall below the association constant of critical enzymes such as the sodium-potassium ATPase and the sodium-calcium ATPase (25). This loss of ATP is most likely associated with its utilization to restore transmembrane ionic gradients that were disrupted by ischemia and hypothermia. Although hypothermia reduces energy requirements and ATP expenditure by slowing metabolic processes, it directly suppresses the ATPase sytem that normally regulates ion distribution across cellular membranes--i.e. ATPase suppression results in potassium and magnesium loss, and sodium and chloride gain by the cell(54).

The increase in intracellular osmotic force passively draws water into the cells. The resultant cellular swelling may obstruct small vessels and worsen the actual ischemic tissue damage(48). In addition, as intracellular potassium levels fall, extracellular calcium enters the cell more readily. Suppression of the normal ATP-dependent sodium-calcium exchange mechanism by cold and/or ischemia or due to a relative deficiency of ATP following reperfusion allows

intracellular calcium to rise to high levels. Eventually myocardial contracture such as is seen with "stone hearts" or "reperfusion injury" may result(9,26).

Unlike standard, extracellular-type media such as saline, Krebs' solution, or Ringers lactate, intracellular-type fluid(ICF) is thought to prevent or reduce such detrimental changes in ATP levels and ion distribution in stored organs. ICF mimicks the normal composition of fluid within cells, especially the high potassium and low sodium concentrations, causing ions to equilibrate across cellular membranes at intracellular concentrations. Therefore, during cold storage in ICF, intracellular potassium and sodium levels will in theory remain unchanged. Normal transmembrane gradients and effective electromechanical function will be readily reestablished upon blood reperfusion. In this way, the use of ICF eliminates the considerable ATP expenditure which would be needed to regenerate transmembrane ionic gradients and diminishes cellular swelling and calcium uptake and the resultant sequelae (9,30).

<u>The Use of ICF for Kidney and Lung Preservation.</u> The use of ICF for enhancement of hypothermic organ preservation was first described by Keeler et al. over twenty years ago (40). Subsequent studies by Collins(1969) and Sacks(1973) convincingly demonstrated the effectiveness of simple bathing in cold ICF to preserve canine kidneys for 30 to 72 hours (15,66). These studies again demonstrated the clear benefit of pharmacologic preservation in addition to the protection

afforded by hypothermia alone. Collins' and Sacks' solutions are still used clinically and make international exchange of renal allografts possible. These solutions are also currently used in clinical lung transplantation, since the only satisfactory results in lung preservation have been obtained with intracellular-type solutions (33,59,73).

ICF and Cardiac Preservation. Clinical use of ICF for human heart transplantation has not yet been attempted. However, a number of investigators have studied intracellular-type fluids for preservation of animal hearts. Kohno et al.(1987) preserved isolated rat hearts for four hours at zero degrees Celsius in a bath of Collins' solution and compared the myocardial cation contents during storage and reperfusion to those in hearts stored in a standard, low potassium-high sodium, cardioplegia. ICF indeed prevented myocardial sodium accumulation and potassium depletion during storage and myocardial sodium and calcium overload after reperfusion compared with the standard cardioplegia (41). This study supports the theory that the benefits of ICF are not solely due to potassium-induced diastolic arrest of the heart.

Feeley et al.(1986) also used Collins' solution for short-term canine heart preservation. This group attempted en bloc heart-lung storage for five hours in a four-degree Celsius bath of Collins' solution. After combined heart-lung transplantation recipient dogs succumbed secondary to pulmonary edema, but cardiac function was adequately

preserved. The cardiac output was also better in transplanted animals following Collins'-storage than after storage with standard cardioplegia (23).

Other investigators have used ICF for intermediate-length (eight to fourteen hours) cardiac preservation with variable success. In 1972, Calman and Bell perfused isolated rat hearts with Collins' solution at five-degrees Celsius for fourteen hours. The hearts were evaluated after reestablishing circulation by connecting them to the aorta and vena cava of an intact "recipient" rat. To be scored as "viable", electrocardiographic evidence of electrical activity was necessary and the heart had to beat continuously for at least ten minutes following reperfusion. Hearts perfused with Collins' solution had very poor viability (<10%) in comparison to hearts perfused with standard cardioplegic solutions containing insulin, 15% dextran-40 and phenoxybenzamine (12).

As part of their study to define the limits of hypothermic preservation, Griepp et al.(1974) stored five canine hearts in a bath of four-degree Celsius Collins's solution for twelve hours. Post-storage function was assessed by orthotopic transplantation with an average survival of 21 hours, reflecting "borderline organ viability" (31). Sewell et al.(1978) achieved much better results by using immunosuppression to evaluate chronic animal survival following orthotopic transplantation. Six canine hearts were transplanted after eight hours of simple refrigeration at

eight-degrees Celsius in Collins' solution. All six recipient dogs survived the perioperative period although atrial pacing and dilute norepinephrine and dopamine drips were required for the initial six to twelve hours. Two dogs died secondary to non-cardiac causes within the first month. The other four dogs survived and underwent cardiac catheterization at one month post-op which demonstrated normal filling pressures, ejection fraction, and cardiac output (68).

Twenty-Four Hour Cardiac Preservation with ICF. Tables 1 and 2 present the composition and experimental design of the previous studies which have used intracellular-type fluids for long-term (24 hour) preservation of canine hearts.

Study	Bayliss (1970)	Reitz* (1974)		Thomas* (1975)		Watson* (1977)		Suzuki (1984)	Present Study (1988)
Solution (approx. composition ** (g/L)	"Fluid II"	C C=Collins' S=Sacks' --=unnamed	S	S	--	--	--	modified Collins'	---
Sodium	30	10	14	14	16	16	16	10	15
Potassium	130	115	126	126	130	117	117	115	130
Calcium	0.1	--	--	--	--	--	--	1.0	--
Magnesium	2	30	16	16	16	60	60	6	3
Chloride	30.1	15	16	16	16	15	15	15	15
Phosphate	100	100	120	120	100	100	100	95	160
Sulfate	2	30	--	--	--	60	60	6	3
Bicarbonate	130	10	20	20	20	20	20	10	20
Glucose(g/L)	--	25	--	--	20	20	20	14	20
Dextrose(g/L)	10	--	--	--	--	--	--	--	--
Dextrose-40 (g/L)	40	--	--	--	--	--	--	--	--
Insulin(u/L)	20	--	--	--	--	--	--	--	--
Methylpred. (mg/L)	125	--	--	--	--	--	--	--	--
Heparin(u/L)	20k	--	--	--	--	--	--	--	
Mannitol(g/L)	--	--	50	50	25	25	25	--	25
Fluorocarbon	--	--	--	--	--	--	--	10%	--
ATP-MgCl2 (m/L)	--	--	--	--	--	--	--	40	--
5% Lidocaine	--	--	--	--	--	--	--	10cc	--
Temperature (deg. Celsius)	0	4	4	4	4	3	3	4	4
Oxygenation	No	No	No	No	No	Yes	Yes	No	No
Osmolarity (mOsm/L)	354	330	430	430	460-480	460	460	410	480

Table 1. Composition of Intracellular-type Solutions used for 24-Hour Cardiac Preservation.

*Investigators using two intracellular-type solutions

**mEq/L except where otherwise specified

Study:	Bayliss (1970)	Reitz (1974)	Thomas (1975)	Watson (1977)	Suzuki (1984)	Present (1988)
N=	10	11/7	12/15	6/6	5	6
Storage Method:	simple bath	simple bath	simple bath	a) perfused b) simple bath	perfused	simple bath
Storage Time:	24 hrs.	18-26 hrs.	24 hrs.	24 hrs.	24 hrs.	24 hrs.
Assess-ment:	gross, ECG	ortho. tx.	hetero. tx., ECG	ortho. tx.	LV balloon ortho. tx.	LV ball.
Temp. (degrees Celsius):	0	4	4	3	4	4

Table 2: Design of 24-Hour Preservation Studies Using ICF

Bayliss and Maloney(1970) were the first investigators to use ICF for cardiac storage. They placed ten canine hearts in a bath of ICF and a second group of control hearts was stored in Normosol (extracellullar-type solution) for twenty-four hours at zero degrees Celsius. The hearts stored in Normosol did not regain an effective beat upon blood reperfusion from a support dog. In contrast, all ten of the hearts stored in ICF "beat vigorously" and regained electrical activity for the full three-hour evaluation period following reperfusion(6).

Reitz et al.(1974) also reported effective twenty-four hour preservation of canine hearts using a simple hypothermic bath of ICF. Reitz evaluated eleven hearts stored in Collins' solution and seven more stored in Sacks' solution by orthotopic transplantation. As evident in table 1, Sacks' solution has approximately the same intracellular ion composition as Collins' solution, but is made hyperosomolar with mannitol. All eighteen hearts were easily defibrillated upon reperfusion with warm blood and supported the circulation once weaned off of cardiopulmonary bypass although eleven required a continuous isoproterenol infusion. Fourteen out of the eighteen recipient animals were weaned from the ventilator and lived eight hours to five and one-half days with four surviving until rejection. Only one of three hearts stored similarly in Krebs' solution could be defibrillated and it did not support the circulation (65).

Thomas et al.(1975) confirmed Reitz's findings with

Sacks ' solution (about 25% survival to rejection) and also described a new solution with even higher osmolarity that yielded a 93% survival rate. The viability of preserved hearts was assessed by gross observation, ventricular pressure measurements, and electrocardiographic tracings following heterotopic transplantation into the neck of recipient dogs. Twelve hearts were stored in a simple bath of Sacks' solution at four-degrees Celsius for twenty-four hours. All were resuscitated but only three (25%) survived until rejection at five to twenty-six days. Fourteeen of the fifteen hearts (93%) stored in Thomas' new, hyperosmolar solution survived beyond six days to rejection (range six to fifty-seven days) (72).

Watson(1977) performed the next studies of cardiac preservation with ICF. He compared simple refrigeration to continuous perfusion using foxhound hearts and orthotopic transplantation. Six donor hearts were perfused continuously at three-degrees Celsius for twenty-four hours with oxygenated ICF. Six other hearts were suspended in a bath of cold, oxygenated ICF. Of the twelve donors, six perfused and four nonperfused grafts survived the twenty-four hour post-transplant period. Two nonperfused grafts died within two hours after weaning from bypass secondary to intractable tachyarrythmias. More severe damage was also noted on gross examination of the nonperfused hearts although they had an average weight loss of 5% in contrast to the 34% average weight gain of the perfused hearts (78).

In a separate study using the same intracellular-type solution, Watson and his colleagues compared continuous to intermittent perfusion for 48-hour preservation. Seventeen foxhound hearts were perfused continuously with oxygenated ICF for 48 hours at three-degrees Celsius. Six others were stored for 48 hours using 15 minute cycles of oxygenated perfusion and non-perfusion. Following orthotopic transplantation, all six(100%) of the intermittently perfused and four(24%) of the continuously perfused hearts survived the 24-hour postoperative period. Several of the recipient animals required ventricular pacing and all of them were continuously supported by dopamine infusions and ventilated postoperatively. The dogs were sacrificed 24 hours after transplantation and there was a significant weight gain for both sets of hearts; 58% for intermittently-perfused and 86% for the continuously-perfused hearts (79).

The only other, and most recent, evaluation of ICF for 24-hour preservation was performed in Japan by Suzuki et al.(1984). Five isolated canine hearts were refrigerated at four-degrees Celsius while a modified Collins' solution was perfused retrogradely into the the coronary sinus. A left ventricular balloon was used to study the hearts following storage and three of them were also assessed by orthotopic transplantation. There was no weight change in the hearts, all five had normal end-systolic pressure-volume ratios, and the three transplanted hearts supported the circulation for a

few hours postoperatively. Long-term survival was not pursued(70).

E. Experimental Design

The present study was undertaken to reevaluate simple, hypothermic, twenty-four hour storage with a fundamental intracellular-type solution. Previous investigators have judged the success of ICF-preservation by subjective, gross observation or by orthotopic transplantation. Although survival of the animal with the orthotopically transplanted heart is a practical endpoint, survival as an "all-or-none" phenomenon does not adequately quantitate graft function. Therefore, a particular objective of this study was to perform precise assessment of hemodynamic and metabolic parameters of ventricular function.

To accomplish this we chose the technique developed by Opie and Gorlin for isolated rat hearts (22,61) and subsequently modified by Holdefer and Edwards(36) for canine hearts. An intraventricular balloon was placed into a basic Langendorf preparation(46) which uses a non-working, isolated heart supported by retrograde coronary perfusion from a support animal. This model allows heart rate and preload to be controlled while another determinant of mechanical performance, the inotropic state is evaluated. It also has the advantage of perfusing the isolated hearts with a biologic medium at the appropriate temperature and level of oxygenation.

Reitz and Thomas achieved successful myocardial preservation using simple immersion of hearts in cold ICF.

For the present study, the convenience of simple storage in ICF was also felt to outweigh the unproven benefits of continuous perfusion,. Our storage solution was designed to be cheap and easy to prepare and store. Therefore, unlike some of the previous ICF studies (Table 7) the solution was kept very fundamental; It was not oxygenated and did not contain other agents such as steroids, fluorocarbons or phenoxybenzamine. The ICF was made hyperosmolar with mannitol and glucose and cooled to four degrees Celsius since, as discussed above, there is a theoretical basis and experimental evidence to support the use of both hyperosmolality and hypothermia in organ preservation.

No attempt was made to compare the efficacy of our ICF with that of any other solution or to compare simple storage to a more complex perfusion system. This study also used each heart as its own control with pre- and post-preservation assessment as has been recommended(50) but only rarely attempted previously(71,74) with isolated hearts.

III. MATERIALS AND METHODS

III. MATERIALS AND METHODS

A. Preparation of the Langendorf Heart

As discussed above, the well-known Langendorf preparation (46) was used to achieve coronary perfusion of the isolated heart from the arterial circuit of a support dog. All experimentation was approved by and conducted under the supervision of the Animal Care Division.

Six mixed-breed dogs weighing 20kg to 25kg were obtained from Paradis Animal Farm (Brookshire, VT). The animals were anesthetized with sodium pentobarbitol (Abbot Laboratories, North Chicago, IL) and maintained on a respirator (Harvard #607, Harvard Apparatus Co., Millis, MA). Anticoagulation was provided with heparin (Elkins-Sinn, Cherry Hill, NJ). The heart was exposed through a median sternotomy and the azygous vein was ligated. The venae cavae, brachiocephalic artery, and the transverse aortic arch were sequentially ligated as 500 milliliters of four-degree Celsius standard operating room cardioplegia (K+ 25 mEq/L) was infused into the coronary arteries through a cannula in the subclavian artery. The inferior vena cava was incised to decompress the right ventricle and iced saline was used for topical hypothermia. The cardioplegia administration brought about rapid hypothermic and metabolic arrest and the heart was then

rapidly excised and placed in an iced cardioplegia bath. A soft latex balloon was connected to a central high pressure tube and was placed in the left ventricular cavity following excision of the mitral valve chordae tendinae. The balloon was mounted on a rubber plug which was held securely in place by a purse-string suture through the mitral valve annulus. The balloons were pre-stretched and their intrinsic distensibility checked to ensure that the material itself made a negligible contribution to the end-diastolic pressure. The stiff rubber plug needed to be positioned carefully so that it would not interfere with the competence of the aortic valve. Retrograde aortic flow from the support dog, if allowed direct access to the left ventricle via a leaky aortic valve, would have invalidated both the balloon and metabolic measurements. In addition, the left atrial remnants were sewn tightly over the rubber plug to ensure that the left ventricle would contract isovolumetrically as the balloon was inflated.

The cannula coming from the balloon emerged from the rubber plug and was connected to a pressure transducer (Gould-Statham, Oxnard, CA) for measurement of left ventricular pressure (LVP). Positive left ventricular dp/dt (LV dp/dt) was obtained by differentiating the LVP signal. Vents made of 3/16th inch Silastic tubing (Dow Corning Corp., Midland, MI) were placed in both ventricles to collect the Thebesian and coronary sinus flow. Epicardial pacing wires were placed on the surface of the heart and connected to a

demand pacemaker (Medtronics, Minneapolis, MN) set to maintain a minimum heart rate of 100 beats per minute. A cannula was placed in the aorta and then attached to a circuit for cross-perfusion from the femoral artery of a support dog (Figure 1). Care was taken to empty all air from the aorta before beginning perfusion. The circuit was primed with 300 milliliters of heparinized blood taken from the donor dog before excision of the heart. The average time to excise the heart, prepare it with the balloon and vents, and to then connect it to the perfusion circuit was 40 minutes. The heart was kept bathed in iced saline as much as technically possible during this preparation.

Following preparation, the isolated heart was cross-perfused retrogradely through the aorta with warm, oxygenated blood from the femoral artery of a heparinized, anesthetized support dog. The coronary artery perfusion pressure was monitored at the aortic root and maintained at 80 to 100 mmHg by controlling the flow from the support dog with a large C-clamp. The drainage from the two vents was collected in a one-liter, water-jacketed chamber (Yale Glassworks Lab, New Haven, CT), maintained at thrity-seven degrees Celsius by a heat exchanger (Travenol Labs, Deerfield Ill.), and returned to the support dog by the femoral vein.

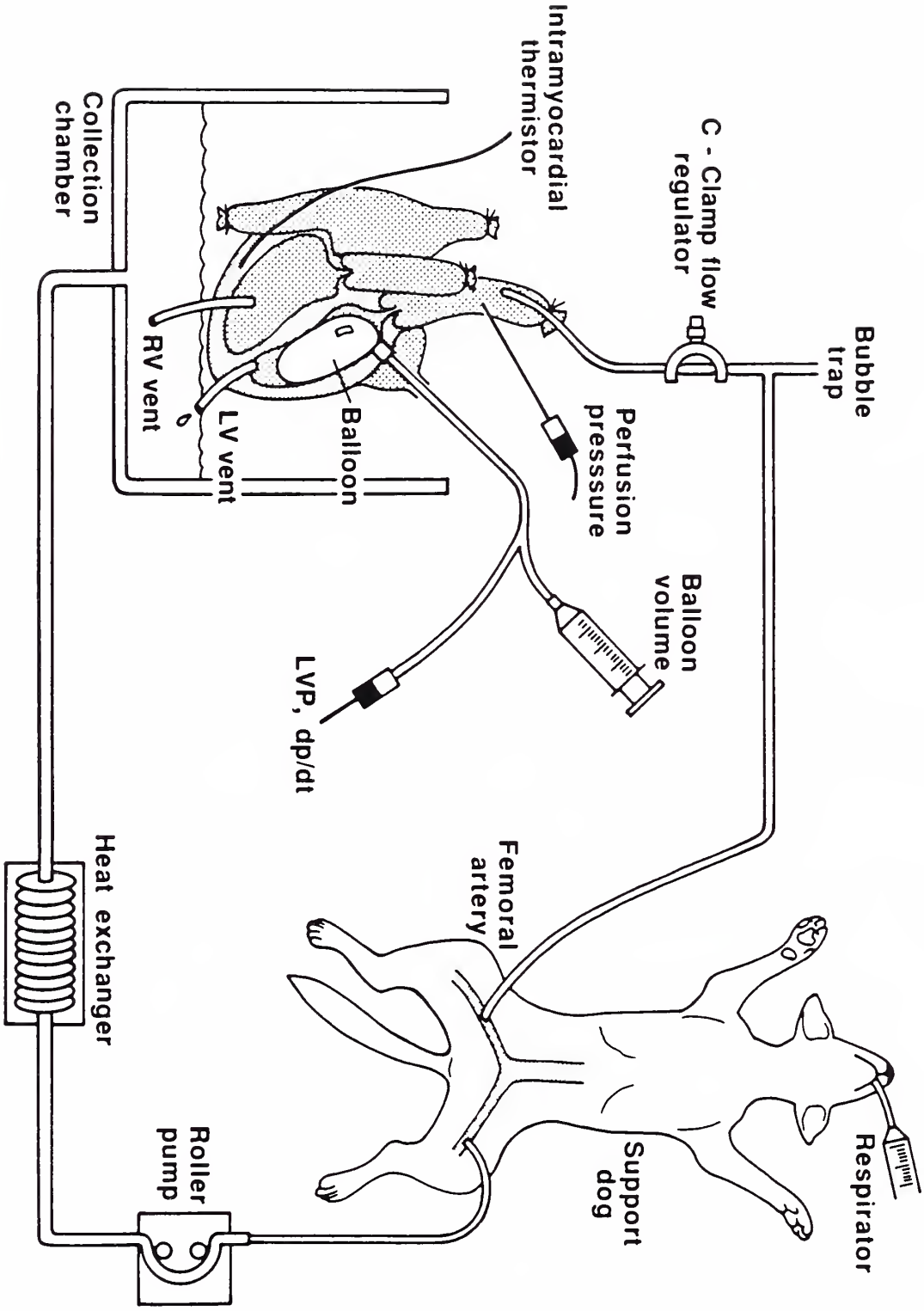

Figure 1: Circuit used for Parabiotic Perfusion of the Isolated Heart

B. Functional Evaluation

Electrical defibrillation was used if defibrillation did not occur spontaneously upon perfusion of the heart in the support circuit. A thirty minute period of stabilization was allowed before baseline (pre- twenty-four hour preservation) data was recorded.

Hemodynamic assessment The isovolumetric left ventricular function was then evaluated by infusion of saline into the left ventricular balloon in five milliliter increments up to twenty milliliters while measuring LVPD and LV dp/dt. Care was taken to prevent air bubbles from entering the intraventricular balloon. During this period of assessment, the heart was kept suspended well above the fluid in the collection chamber, since submersion changed the transmural pressure and influenced the left ventricular pressure tracings. The optimal balloon volumes were determined during the initial preservation experiment. Figure 2 illustrates that volumes of five to twenty milliliters clearly place this heart on the ascending portion of the "Starling Curve" of ventricular function. These balloon volumes are in accordance with those used in other studies of isolated canine hearts (7,70).

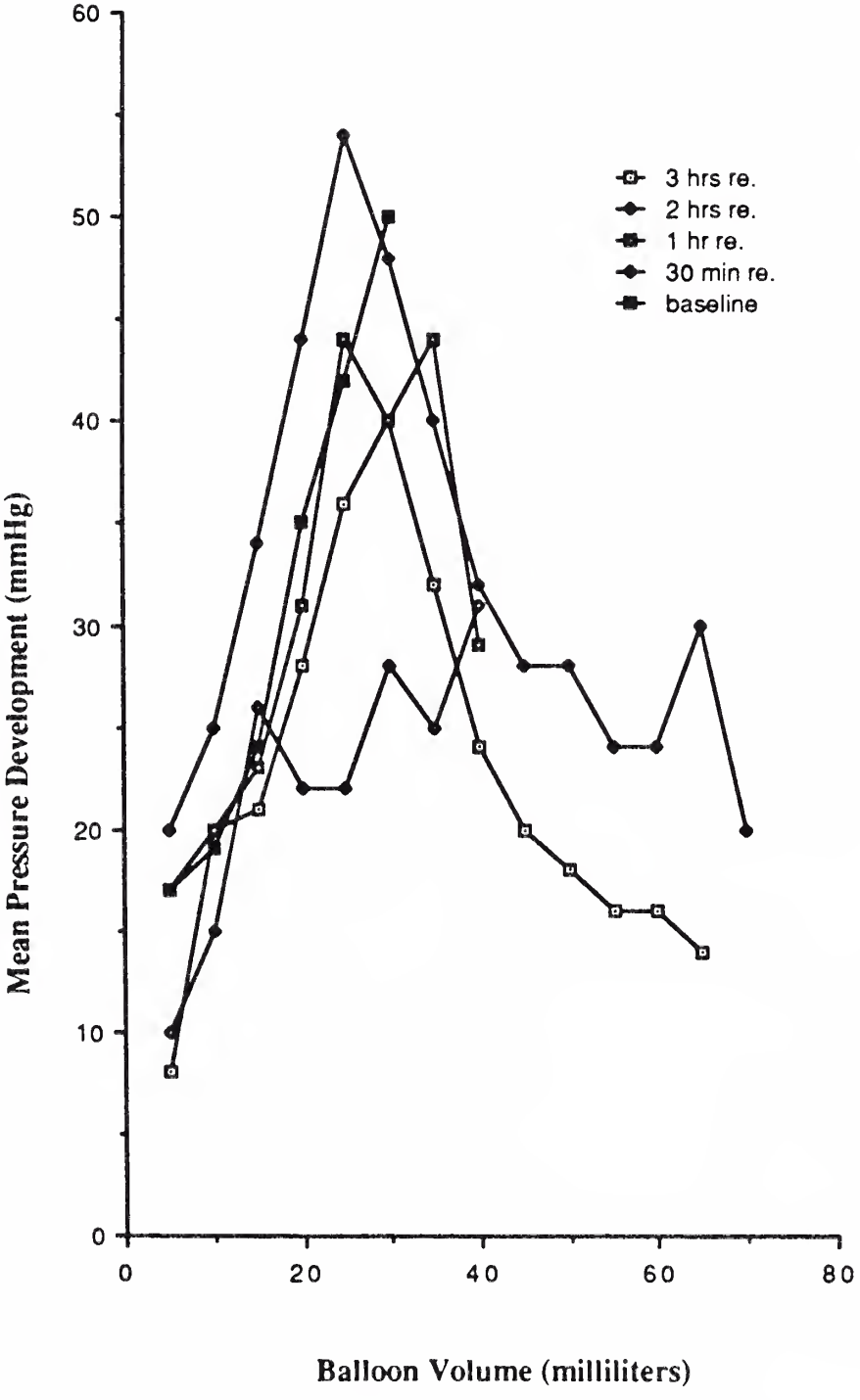

Figure 2: Pressure Development Versus Balloon Volume (Expt. #1)

Metabolic assessment and determination of water content

Myocardial water content was determined by weight difference before and after drying biopsies of left ventricular muscle to constant weight. Control water content was determined similarly from samples from freshly excised hearts. Arterial and coronary sinus lactate levels were determined to allow calculation of lactate extraction: [(arterial lactate - coronary sinus lactate)/(arterial lactate)]. Oxygen content (volume %) was also measured in arterial and coronary sinus blood to allow calculation of oxygen extraction: [(arterial oxygen content - coronary sinus oxygen content)/ (arterial oxygen content)]. Coronary sinus drainage from the right ventricular vent (ml/min) was multiplied by the arterial-coronary sinus oxygen difference to yield values for myocardial oxygen consumption.

C. Preparation of Intracellular Fluid and Twenty-Four Hour Storage

After obtaining the baseline data, the intraventricular balloon was emptied and the isolated heart was again rapidly arrested, this time by flushing approximately 100cc of four-degree Celsius intracellular-type fluid (ICF) into the coronary arteries as the blood flow from the support dog was interrupted. When the coronary sinus flow became clear, the heart was completely submerged in a one-liter bath of cold, unoxygenated ICF and placed into a refrigerator. A thermistor was placed into the left ventricular chamber via the LV vent and the temperature was maintained at four-degrees Celcius throughout the twenty-four hour storage period.

The ICF was modelled after other intracellular-like fluids with high osmolarity which have been studied previously (see Table 1 above). The recipe (Table 3), yielded the ionic concentrations noted in Table 4 as determined by the clinical chemistry laboratory.

A relatively low concentration of magnesium was used since, as noted in previous studies also (5,65), magnesium phosphate crystals tended to form spontaneously when more magnesium sulfate was added. Such a precipitate could be detrimental if it were to occur in the coronary vasculature

Potassium phosphate monobasic	2.05
Potassium phosphate dibasic	9.70
Potassium chloride	1.12
Potassium bicarbonate	2.30
Sodium bicarbonate	1.25
Magnesium sulfate hydrate	7.40
Glucose	20.0
Mannitol	25.0

Table 3. Recipe for Intracellular Fluid (gm/L)

during storage of the hearts.

The ICF was prepared separately for each experiment. Although the compounds used are all quite water soluble (82), each was brought into solution completely using double-distilled water before the next compound was added. The compounds were added in the order listed in Table 3. Careful preparation of the ICF in this manner was necessary to prevent insoluble complex ions, such as magnesium phosphate tribasic, from forming secondary to locally high ion concentrations. The ICF was refrigerated at four-degrees Celsius overnight and was then filtered through a 22 micron Whatman filter prior to use. The ICF in the storage bath was replaced with fresh solution after eight and again after sixteen hours of storage.

D. Blood Reperfusion

After twenty-four hours of hypothermic storage in the ICF bath, the heart was rewarmed by cross-perfusion from a support dog and again electrically defibrillated when the myocardial temperature reached thirty degrees Celsius. During the initial period of reperfusion, all coronary venous effluent was discarded. The function tests performed during the baseline evaluation were repeated 0.5, 1.0, 2.0, and 3.0 hours following restoration of supraventricular rhythm. During both pre- and post-storage functional evaluations, the arterial blood gases, pH, hematocrit, and arterial blood pressure of the support dog were monitored and maintained within normal physiologic range. No inotropic agents were used to stimulate the support dog or the isolated hearts at any point.

Potassium	130 mEq/L
Sodium	15 mEq/L
Magnesium	3 mEq/L
Chloride	15 mEq/L
Phosphate	160 mEq/L
Bicarbonate	30 mEq/L
Sulfate	3 mEq/L
Osmolarity	480 mOsm/L
pH	7.2 at 4 C

Table 4. Composition of Intracellular Fluid

E. Handling of Hemodynamic Data

All values of left ventricular pressure development (LVPD) and left ventricular positive dp/dt (LV dp/dt) were extracted from the chart recorder tracings as averages for ten consecutive heartbeats. The LVPD for a single cardiac cycle was calculated as the end-systolic left ventricular pressure minus the end-diastolic left ventricular pressure. For each heart, post-preservation values of LVPD and LV dp/dt at each time point were expressed as a percentage of baseline values for that heart at all four balloon volumes. These normalized values were then averaged to determine the percent-baseline function for that heart at each time point. The overall adequacy of twenty-four hour preservation was judged by compiling the percent-baseline data for all six hearts for these hemodynamic parameters.

Diastolic compliance was measured at baseline and at each reperfusion time point. Compliance was calculated as deltaV/deltaP for the end-diastolic pressures as the balloon was inflated from five to twenty milliliters.

F. Statistical Analysis

All results are presented as means plus/minus the standard error of the mean. The mean values obtained following preservation were compared with the baseline data using the paired Student's T-test with differences considered significant for values of $p < 0.05$.

The diastolic compliance curves were fitted to a straight line by linear regression using the least-squares method, with a coefficient of determination (r-squared) greater than 0.98 in all cases.

IV. RESULTS

IV. RESULTS

A. Pressure Development and Contractility

Upon blood reperfusion all six hearts stored for twenty-four hours were easily electrically defibrillated at a temperature of thirty degrees Celsius and maintained a supraventricular rhythm at a rate of 100 to 140 beats per minute throughout the three-hour period of evaluation. Five out of six hearts beat regularly without significant arrythmias. The sixth heart had a persistent irregularly irregular rhythm which interferred with the functional assessment using the intraventricular balloon. However, when a bolus of lidocaine was given to the support dog, periods of regular rhythm resulted which facilitated the data collection. At the end of all six experiments, the latex balloon was found to conform uniformly to the walls of the left ventricular chamber.

As noted above, balloon volumes of five to twenty mililiters represented a "preload" that placed the hearts on the ascending portion of the ventricular function curve. As shown for the first experiment, Figure 2, at balloon volumes greater than thirty mililiters ventricular function was depressed. At much higher volumes the left ventricle was visibly distended and beating weakly and the perfusion

pressure at the aortic root was greatly increased. The LV dp/dt for this heart showed similar response to changes in balloon volume (Figure 3). Furthermore, overdistension of the left ventricle following two hours of blood reperfusion, resulted in significantly poorer subsequent function.

Table 5 contains the average values of LVPD and LV dp/dt for the six hearts at a balloon volume of twenty milliliters. These values put the levels of percent-baseline function determined for all four balloon volumes (Table 6, Figures 6,7) into perspective.

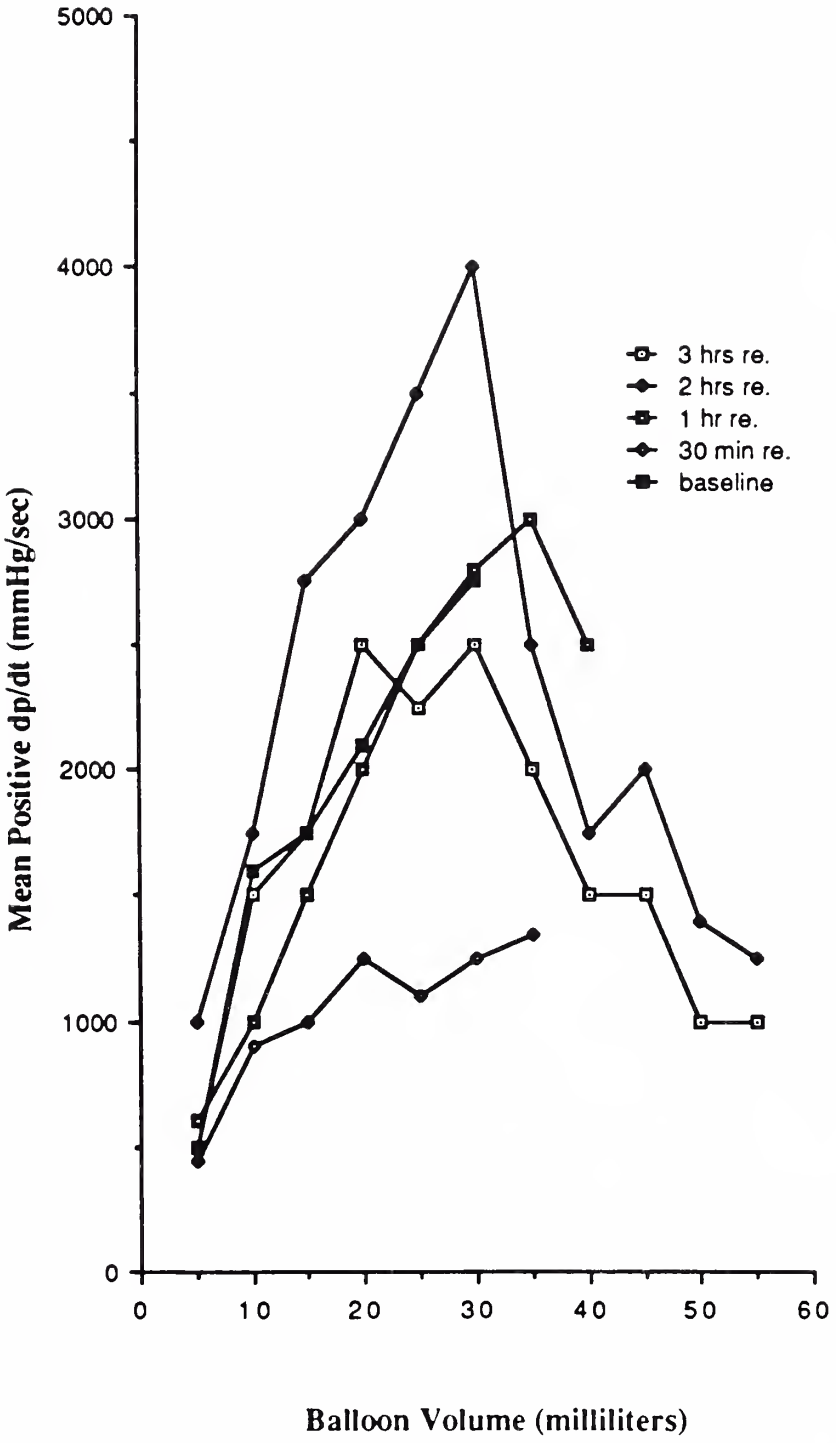

Figure 3: Positive dp/dt Versus Balloon Volume (Expt. #1)

	Mean LVPD (mmHg, N=6)	Mean LV dp/dt (mmHg, N=6)
Baseline	89 ± 13	2750 ± 537
30 Minutes Reperfusion	39 ± 5	1788 ± 377
60 Minutes Reperfusion	54 ± 10	2300 ± 551
2 Hours Reperfusion	72 ± 10	4021 ± 1060
3 Hours Reperfusion	69 ± 12	4000 ± 1318

Table 5. Average LVPD and LV dp/dt at a balloon volume of 20 milliliters

Figure 4 illustrates the chart recorder data obtained for a representative, twenty-four hour preserved heart after three hours of blood reperfusion (~ 28 hours post excision) at a balloon volume of twenty mililiters. Figure 5 contains the ventricular function curves, LVPD versus balloon volume, for the same representive heart. Left ventricular function was significantly depressed after thirty minutes of reperfusion and improved progressively with LVPD approaching this heart's baseline after two hours of reperfusion.

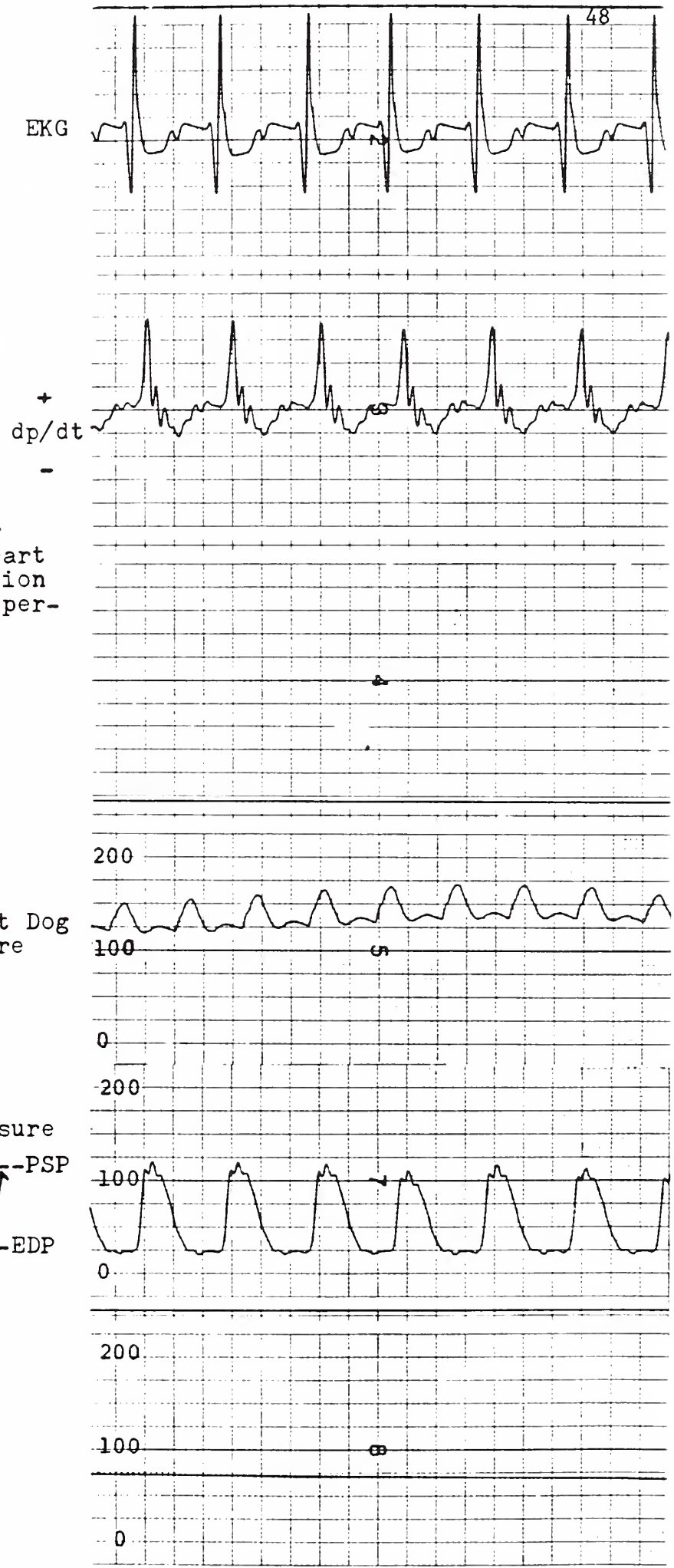

Figure 4: Chart recorder data for an isolated heart after 24-hour preservation and 3 hours of blood reperfusion (LVPD-Left Ventricular Pressure Development)

Perfusion Pressure (Measured @ Aortic Root)

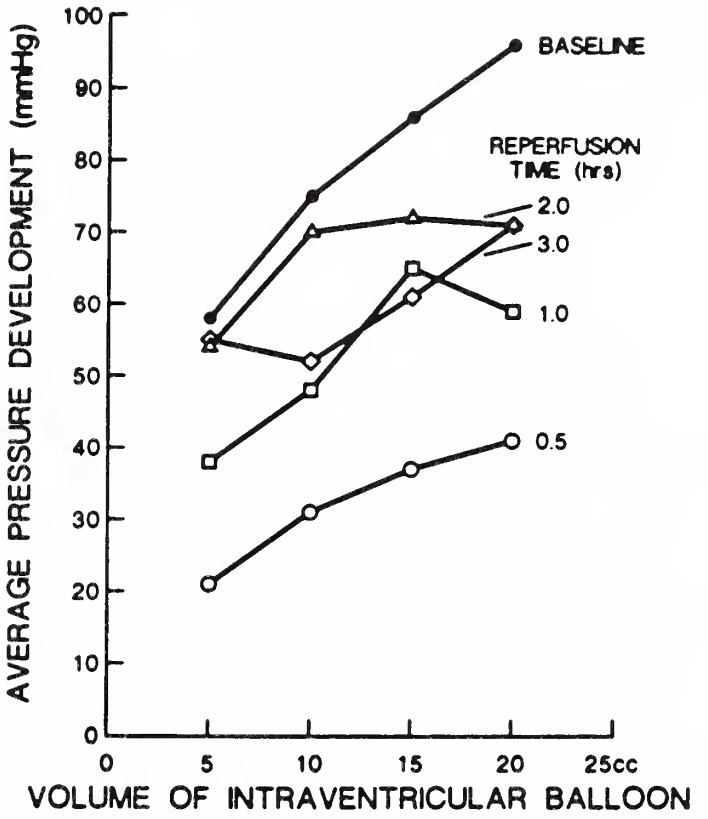

Figure 5: Ventricular function curves for a representative isolated heart at baseline and after twenty-four hours of preservation and blood reperfusion

The general trend of hemodynamic recovery during reperfusion, demonstrated by the single heart above, was observed for all six hearts. The composite hemodynamic data for the six hearts is presented in Table 6 and graphically in figures 6 and 7. Ventricular pressure development and positive dp/dt were both significantly depressed thirty minutes after the start of reperfusion. Both LVPD and LV dp/dt increased during the next several hous and were not statistically different from baseline after two hours of reperfusion.

Heart no. 1, see Figures 2 and 3, apparently sustained an air embolus during the start of perfusion for its baseline evaluation. This probably accounts for the smaller absolute values of pressure development and LV dp/dt seen for this heart. Nonetheless, this heart also demonstrates the general trend of hemodynamic recovery upon reperfusion until it was injured by overdistension at the two-hour reperfusion time point.

	%Baseline LVPD	%Baseline LV dp/dt
Baseline	100	100
30 Minutes Reperfusion	48 ± 6	45 ± 10
60 Minutes Reperfusion	64 ± 8	66 ± 12
2 Hours Reperfusion	90 ± 8	122 ± 25
3 Hours Reperfusion	78 ± 6	116 ± 25

Table 6. Percent-Baseline LVPD and LV dp/dt Averaged for all Balloon Volumes (N=6)

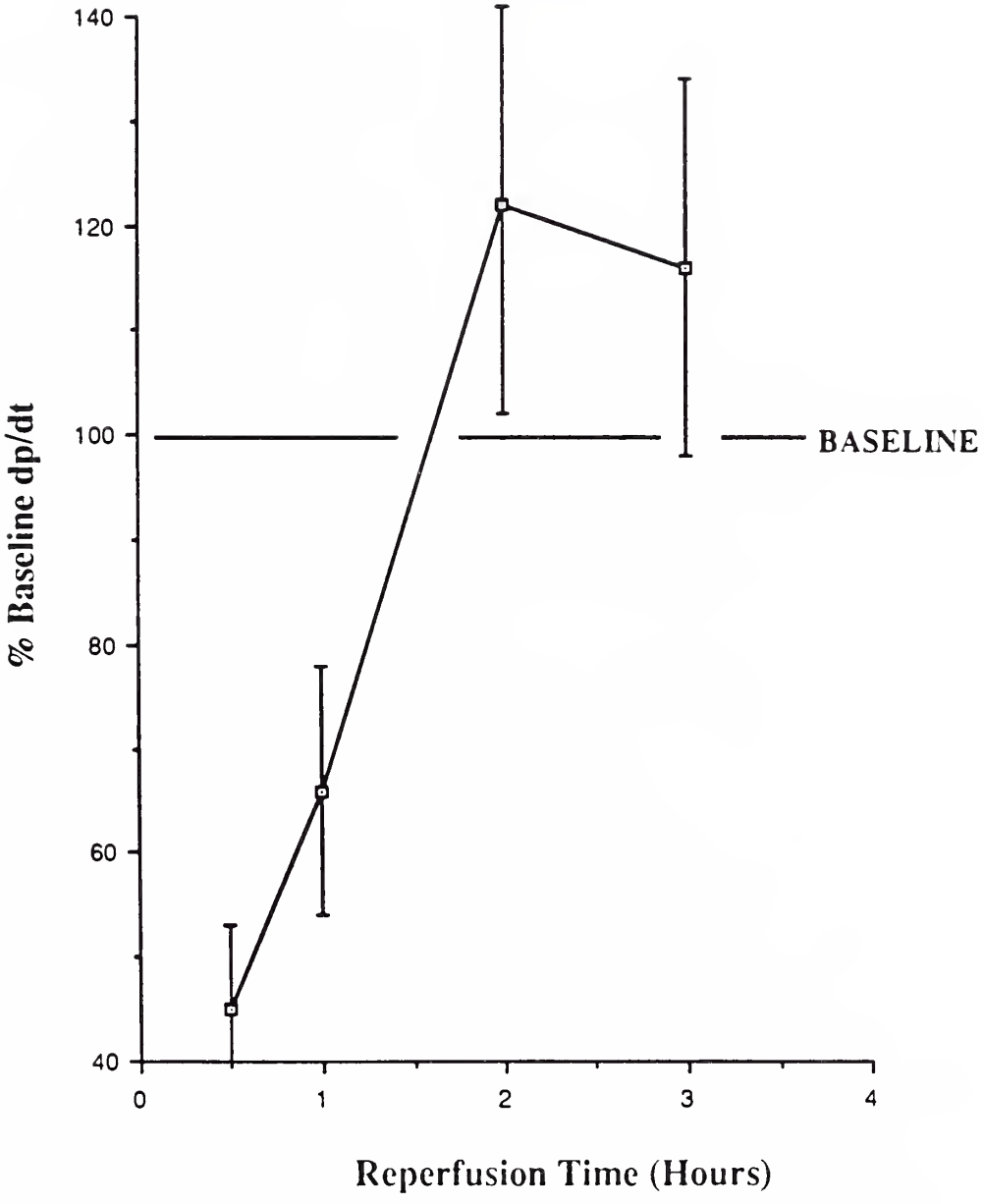

Figure 6: Recovery of dp/dt Following 24-Hour Preservation (N=6)

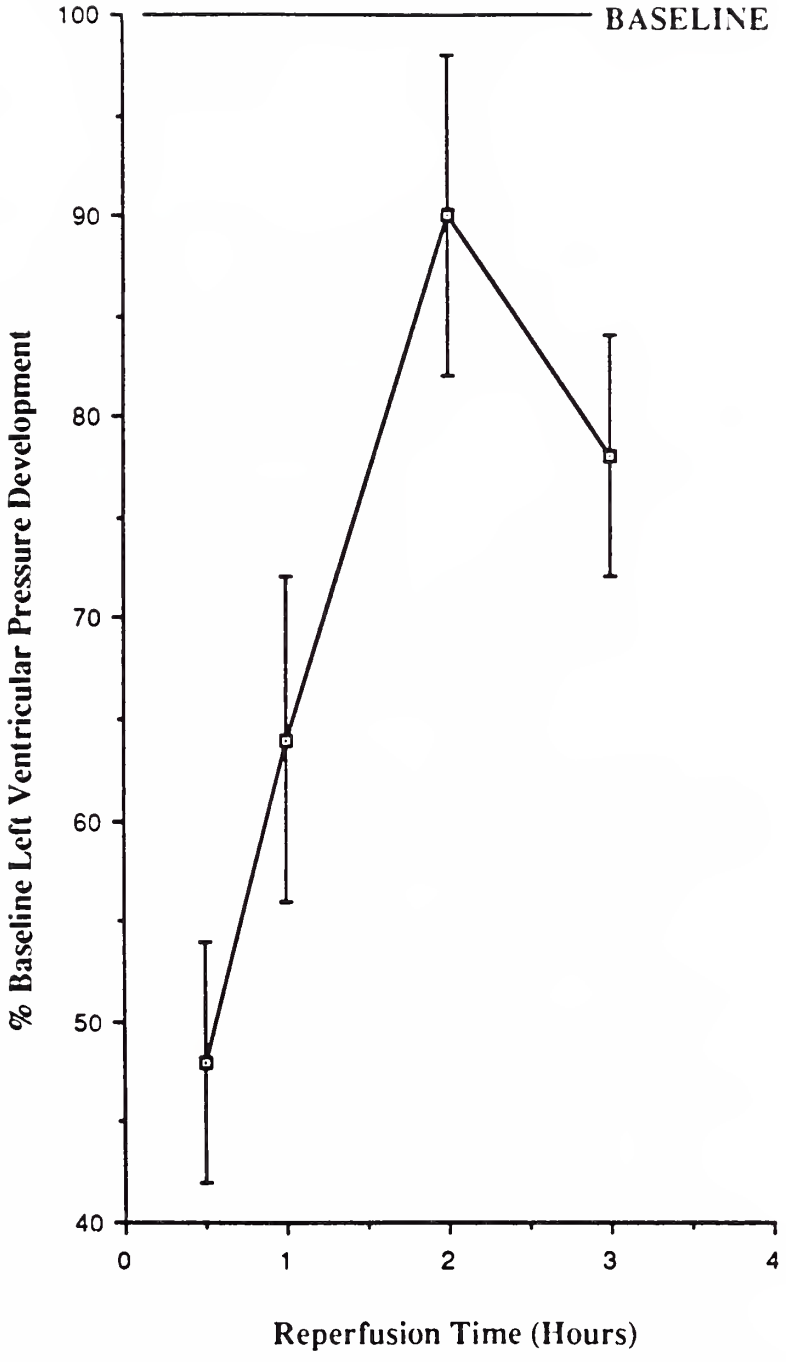

Figure 7: Recovery of Left Ventricular Pressure Development (N=6)

B. Myocardial Compliance and Water Content (Table 7)

The average diastolic compliance after storage and thirty minutes of reperfusion decreased considerably to 0.50 ml/mmHg ± 0.04 from a baseline of 1.9 ml/mmHg ± 0.26 ($p<0.05$). There was no further change at the subsequent reperfusion time points with a compliance of 0.52 ml/mmHg ± 0.03 after three hours of reperfusion. There was a concomitant increase in gravimetric heart water from a control value of 78.7 ± 0.03 ml/100 grams for freshly excised hearts to 82.1 ± 0.4 ml/100 grams for hearts after storage and three hours of blood reperfusion ($p<0.05$).

	Compliance (ml/mmHg)	Water Content (ml/100gm)
Baseline	1.90 ± 0.26	78.7 ± 0.3
30 Minutes Reperfusion	0.50 ± 0.04	----------
60 Minutes Reperfusion	0.55 ± 0.04	----------
2 Hours Reperfusion	0.52 ± 0.03	----------
3 Hours Reperfusion	0.52 ± 0.03	82.1 ± 0.4

Table 7. Diastolic Complicance and Water Content

C. Metabolic Function(Tables 8 and 9)

Lactate extraction was seen in all six hearts during the baseline evaluation. In contrast, all six hearts were producing lactate after storage and thirty minutes of reperfusion. During the next two hours the six hearts tended to increase their lactate extraction and three of them gave evidence of aerobic metabolism by consuming lactate from the arterial blood (positive lactate extraction ratios) after three hours of reperfusion. The average lactate extraction ratio at three hours of reperfusion was not statistically different from baseline. Likewise, the myocardial oxygen consumption and oxygen extraction ratios during the reperfusion period did not differ significantly from the baseline ratios. In addition, the support dogs' femoral artery lactate concentration at baseline (1.3 mmol/L $\pm$ 0.3) was not significantly different from the average value during the reperfusion studies (1.5 mmol/L $\pm$ 0.5 at 30 minutes reperfusion, 1.2 mmol/L $\pm$ 0.3 at three hours reperfusion).

Baseline	0.18 ± 0.01
30 Minutes Reperfusion	-0.60 ± 0.37
60 Minutes Reperfusion	-0.32 ± 0.09
2 Hours Reperfusion	-0.16 ± 0.05
3 Hours Reperfusion	-0.06 ± 0.06

Table 8. **Lactate Extraction Ratios (N=6)**

	Oxygen Extraction Ratio	Oxygen Consumption
Baseline	0.28 ± 0.06	2.8 ± 0.6
30 Minutes Re.	0.44 ± 0.06	2.7 ± 0.5
60 Minutes Re.	0.34 ± 0.03	2.5 ± 0.6
2 Hours Re.	0.47 ± 0.09	3.5 ± 0.7
3 Hours Re.	0.42 ± 0.07	2.8 ± 0.7

Table 9. Oxygen Extraction Ratios and Myocardial Oxygen Consumption

V. DISCUSSION

V. DISCUSSION

Cardiac transplantation for patients with end-stage cardiac disease is currently limited by a natural constraint--the availability of donor organs. Long-term mechanical circulatory support and successful, socially-acceptable xenografting are unlikely to supercede the use of human heart transplantation in the reasonably near future. Therefore, the development of a clinically effective method of long-term cardiac preservation is needed if the shortage of donor hearts is going to be alleviated.

This study was designed to reevaluate the use of simple bathing in cold intracellular fluid (ICF) for twenty-four hour cardiac preservation. Previous studies with simple ICF storage evaluated cardiac function subjectively or by transplantation. We used an isolated, "non-working" heart with isovolumetric contraction on an intraventricular balloon. Each heart's own post-preservation function was compared to its own baseline (pre-storage) function. The intraventricular balloon allowed precise measurement of the first derivative of the ventricular pressure curve (LV dp/dt) which has been shown to be a good measure of inotropic state in experimental animals(77). Left ventricular pressure development (LVPD) was also readily determined with the intraventricular balloon and demonstrated the important

ability of the heart to generate pressure independently above the diastolic "filling pressure" presented by the inflated balloon.

The comparison to baseline LV dp/dt and LVPD (Table 6, Figures 6 and 7) demonstrates that hyperosmolar ICF afforded excellent twenty-four hour preservation. Function was clearly transiently depressed after storage, but adequate function was regained following two hours of blood reperfusion. Not surprisingly, improvement in contractile performance corresponded with a return of metabolic function towards baseline aerobic metabolism as evidenced by positive lactate extraction. The oxygen consumption after storage also compares well with Levitsky's reported values following twenty-four hour preservation with canine plasma(50). The steady femoral artery lactate concentrations at baseline and during reperfusion suggest that the support dogs' metabolic state did not influence the performance of isolated hearts.

The mean LV dp/dt of 2750 ± 537 at baseline compares favorably with previously reported control values of 2960± 180(47) and 1310 ± 109(50) which were also obtained with the intraventricular balloon technique. In a non-ejecting, isolated heart the clinical significance of these values is difficult to interpret. However, they are also similar to previous <u>in vivo</u> control values of 1550 ± 220, 2000 ± 590 mmHg/sec(44) and 1275 mmHg/sec(57). This suggests that the six preserved hearts in this study recovered to a contractile state that would meet physiologic demands. The average LVPD

of 69 mmHg following twenty-four hour storage and three hours of blood reperfusion also reflects the integrity of contractile function to within a normal physiologic range. Following clinical transplantation, positive inotropic agents would be available to further enhance cardiac performance.

The baseline values of myocardial water content agree very well with previously reported control values(47,42). The baseline value for diastolic compliance, 1.90 ± 0.26 ml/mmHg, is virtually identical to a previous *in vivo* control value of 1.95 ± 0.30 ml/mmHg(44). The post-preservation weight gain and decreased compliance (82.1 ± 0.4 ml/100gm and 0.52± 0.03 ml/mmHg respectively) are also in agreement with prior studies. Laks and Bethencourt reported values of 82.8 ± 0.32 ml/100gm and 0.65± 0.14 ml/mmHg after twenty-four hour storage in modified Krebs solution and three hours of reperfusion(7). Kolata et al. reported a water content of 82.3 ± 0.8 ml/100gm after twenty-four hour storage in a standard extracellular-type solution(42).

Without further measurements at intermittent time points it is impossible to correlate the compliance changes with changes in water content. Since compliance remains unchanged throughout the reperfusion period as ventricular contractility improves markedly, decreased compliance does not appear to prevent good hemodynamic function. This confirms the poor relationship between compliance and hemodynamic function noted by previous investigators (7,80). Furthermore, survival following transplantation is not

predicted well by the weight gain during preservation (17).

In light of the encouraging results achieved in this study further laboratory investigation of ICF seems warranted. While the isolated-heart model provides precise hemodynamic evaluation, and has been used extensively in the literature, it has several inherent shortcomings. It provides no certainty that function of the preserved heart would be as good in the "working" state and in vivo evaluation with precise hemodynamic measurements would be the ultimate test of ICF's clinical utility. In addition, the function of the preserved heart was studied only for the brief three-hour period. It would be important to confirm that good hemodynamic function continues.

A short-term orthotopic transplantation study with full hemodynamic assessment would confirm function of the "working" heart and would allow clinically meaningful parameters such as cardiac output and ejection fraction to be measured. Subsequently, a long-term survival study with precise hemodynamic assessment initially and at intervals would confirm hemodynamic function is sustained. It would also provide the opportunity to see if edema resolves and diastolic compliance increases. Previous studies have indicated that myocardial ultrastructure may be damaged before functional deterioration is evident by routine measurements of contractility (10,53). Therefore, histological studies during ICF preservation would be informative. In addition, since high concentrations of potassium are

extremely irritating to peripheral veins, further studies of its effect on the coronary vasculature are needed. The effect of ICF on cardiac antigenicity also needs to be evaluated.

It is unclear why earlier studies with ICF yielded inconsistent results. The present study was carefully designed to take full advantage of the theoretical benefits of ICF in an attempt to optimize preservation. ICF is felt to prevent myocardial damage during hypothermic storage and electromechanical arrest with high extracellular potassium by maintaining the integrity of transmembrane ionic gradients. Therefore, during rewarming and reperfusion the cells do not need to expend ATP to reestablish the normal ionic gradients and transmembrane voltage potential. The ATP which is saved might then be available to meet other needs of the cell including actin-myosin interaction.

Measurement of ions, including calcium, and ATP levels during the various stages of preservation and reperfusion might demonstrate whether or not ICF works in this manner. Increasing the magnesium concentration to a true intracellular level of about fifteen mEq/L might also improve preservation. Magnesium occurs in the myocardium chelated to ATP and it is also lost from the cell during ischemia or reperfusion. Since magnesium functions, in part, to inhibit mitochondrial uptake of calcium, its loss is felt to be associated with irreversible damage in post-ischemic myocardium (26,58). The speed of rewarming following cold storage might also be important, but

was not investigated in this study.

Lastly, the technique of cardiac preservation investigated--simple immersion in cold ICF-- is so simple that transition to clinical practice would be straightforward. Cold ICF may also prove to be advantageous for short-term (four to six hours) preservation or as operating-room cardioplegia.

VI. SUMMARY

VI. SUMMARY

This study confirms the favorable results that have been achieved using simple storage of hearts in hypothermic, hyperosmolar intracellular-type fluid (ICF). It also demonstrates that it is possible to use each heart as its own control in an isolated, "non-working" model. We conclude that:

1. Twenty-four hour preservation by the simple technique of immersion in cold ICF effectively preserves hemodynamic function as manifested in both pressure development (LVPD) and "contractility" (LV dp/dt). This was accomplished without the use of inotropic support;
2. Hemodynamic function is depressed initially after twenty-four hour preservation and subsequently improves progressively with blood reperfusion, reaching pre-preservation levels after three hours;
3. Recovery of hemodynamic function occurs despite decreased diastolic compliance and the presence of myocardial edema; and
4. Recovery of hemodynamic function corresponds with the return of metabolic function towards baseline.

VII. BIBLIOGRAPHY

VII. BIBLIOGRAPHY

1. Annas, G.J., "No cheers for temporary artificial hearts", Hastings Center Report, 1985(Oct.): 27.

2. Angell, W.W., Rikkers, L., Dong, E., Shumway, N.E., Organ viability with hypothermia. J. Thorac. Cardiovasc. Surg., 1969, 58(5): 619.

3. Barsamian, E.M., Jacob, S.W., Collins, S.C., Owen, O.E., The transplantation of dehydrated and supercooled hearts. Surg. Forum, 1959, 10: 100.

4. Baumgartner, W.A., Reitz, B.A., Oyer, P.E., Stinson, E.B., Shumway, N.E., Cardiac homotransplantation. Curr. Probl. Surg., 1979, 16(9): 2.

5. Bayliss, C.E., (Letter to editor), J. Thorac. Cardiovasc. Surg., 1985, 90: 936.

6. Bayliss, C.E., Maloney, J.V., Shelf-storage of excised hearts by manipulation of transmembrane ionic gradients. Surg. Forum, 1970, 21: 194.

7. Bethencourt, D.M., Laks, H., Importance of edema and compliance changes during 24 hours of preservation of the dog heart. J. Thorac. Cardiovasc. Surg., 1981, 81: 440.

8. Billingham, M.E., Baumgartner, W.A., Watson, D.C., Reitz, B.A., Masek, M.A., Raney, A.A., Oyer, P.E., Stinson, E.B., Shumway, N.E., Distant heart procurement for human transplantation: ultrastructural studies. Circulat., 1980: 62(Suppl.I): 11.

9. Buckberg, G.D., A proposed "solution" to the Cardioplegic controversy, J. Thorac. Cardiovasc. Surg., 1979, 77(6): 803.

10. Buja, L.M., Levitsky, S., Ferrans, V.J., Roberts, W.C., Morrow, A.G., Souther, S.G., Acute and chronic effects of normothermic anoxia on canine hearts: light and electron microscopic evaluation. Circulat., 1971, 43(Suppl.I): 44.

11. Burt, J.M., Copeland, J.G., Myocardial function after preservation for 24 hours. J. Thorac. Cardiovasc. Surg.,1986, 92: 238.

12. Calman, K.C., Bell, P.R.F., Experimental organ preservation. Brit. J. Surg., 1972, 59(10): 758.

13. Christopherson, L. Heart transplants. Hastings Center Report, 1982, 12: 21.

14. Cleveland, R.J., Lower, R.R. Transplantation of canine cadaver hearts after short-term preservation. Transplant.,1967, 5: 904.

15. Collins, G.M., Bravo-Shugarman, M., Terasaki, P.I., Kidney preservation for transportation., The Lancet, 1969, Dec.:1219.

16. Cooper, D.K., Wicomb, W.N, Rose, A.G., Barnard, C.N. Orthotopic allotransplantation and autotransplantation of the baboon heart following 24 hours' storage by a portable hypothermic perfusion system. Cryobiol., 1983, 20: 385.

17. Copeland, J., Jones, M., Spragg, R., Stinson, E.B., In vitro preservation of canine hearts for 24 to 28 hours followed by successful orthotopic transplantation. Ann Surg., 1973, 178: 687.

18. Emery, R.W., Cork, R.C., Levinson, M.M., Riley, J.E., Copeland, J., McAleer, J., Copeland, J.G., The cardiac donor: a six-year experience. Ann. Thorac. Surg., 1986, 41: 356.

19. Engleman, R.M., Rousow, J.H., Dobbs, W.A. Fluosol DA, an artificial blood for total cardiopulmonary bypass. Ann. Thorac. Surg., 1981, 32: 529.

20. Evans, R.W., Manninen, D.L., Gersh, B.J., Hart, L.G., Rodin, J., The need for and supply of donor hearts for transplantation. J. Heart. Transplant., 1984, 4(1): 57.

21. Evans, R.W., Manninen, D.L., Garrison, Jr., L.P., Maier, A.M., Donor availability as the primary determinant of the future of heart transplantation. J.A.M.A., 1986, 255(4); 1892.

22. Fallen, E.L., Elliott, W.C., Gorlin, R., Apparatus for study of ventricular function and metabolism in the isolated perfused rat heart. J. Applied Physiol., 1967, 22: 836.

23. Feeley, T.W., Mihm, F.G., Downing, T.P., Sadeghi, A.M., Baumgartner, W.A., Reitz, B.A., Shumway, N.E., Hypothermic preservation of the heart and lungs with Collins' solution: effect on cardiorespiratory

function following heart lung allotransplantation in dogs., Ann. Thorac. Surg., 1986, 41: 301.

24. Feemster, J.A., Lillehei, R.C.: Hypothermic-hyperbaric pulsatile perfusion for preservation of the canine heart. Transplant. Proc., 1969, 1: 138.

25. Feinberg, H., Levitsky, S., "Biochemical rationale of cardioplegia", in A Textbook of Clinical Cardioplegia, Engleman, R.M., and Levitsky, S. (editors), 1982, Mt. Kisco, NY: Futura Publishing Co., Chapt. 10.

26. Fiskum, S., Lehninger, A.L., The mechanisms and regulation of mitochondrial calcium transport. Fed. Proc., 1980, 39: 2432.

27. Flores, J., Dibuna, D.R., Beck, C.H., Leaf, A. The role of cell swelling in ischemic renal damage and the protective effect of hypertonic solute. J. Clin. Invest., 1972, 51: 118.

28. Foreman, J., Pegg, D.E., Armitage, W.J., Solutions for preservation of the heart at zero-degrees Celcius., J. Thorac. Cardiovasc. Surg., 1985, 89: 867.

29. Gordon, R.T., Myocardial preservation (letter to editor), Ann. Thorac. Surg., 1987, 43(2): 240.

30. Green, C.J., Pegg, D.E., Mechanism of action of "intra-cellular" renal preservation solutions. World Journal of Surgery, 1979, 3: 15.

31. Griepp, R.B., Stinson, E.B., Angell, W.W., Dong, E., Shumway, N.E., Hypothermic preservation of the canine heart. Transplant. Proceed., 1974, 6(3): 315.

32. Guerraty, A., Alivizatos, P., Warner, M., Hess, M., Allen, L., Lower, R., Successful orthotopic canine heart transplantation following 24 hours of in vitro preservation. J Thorac. Cardiovasc. Surg., 1981, 82: 531.

33. Haverich, A., Scott, W.C., Jamieson, S.W., Twenty years of lung preservation--a review. J. Heart Transplant., 1985, 4: 234.

34. Hearse, D.J., Stewart, D.A., Braimbridge, M.V., The additive effects of hypothermia and chemical cardioplegia during ischemic cardiac arrest in the rat. J. Thorac. Cardiovasc. Surg., 1980, 79: 39.

35. Hicks, G.L., Arnold, W., Dewall, R.A. Fluorocarbon cardioplegia and myocardial protection. Ann. Thorac. Surg., 1983, 35: 506.

36. Holdefer, W.F., Edwards, W.S., Ventricular performance characteristics in a metabolically supported isolated heart preparation: a model for evaluating cardiac storage methods., Surg. Forum, 1968, 19: 225.

37. Jynge, P., Hearse, D.J., de Leiris, J., Feuvray, D., Braimbridge, M.V., Protection of the ischemic myocardium. J. Thorac. Cardiovasc. Surg., 1978, 76: 2.

38. Kanter, K.R., Jaffin, J.H., Ehrlichman, R.J., Flaherty, J.T., Gott, V.L., Gardner, T.J. Superiority of perfluorocarbon over blood and crystalloid cardioplegia. Circulat., 1980, 62(3): 323.

39. Katz, A.M., A basic scientist reacts to the discussions of surgery, Proceedings of Cardioplegia Workshop--An International exchange of Ideals, Deerfield, Ill., 1980, Travenol Laboratories, 31.

40. Keeler, R., Swinney, J., Taylor, R.M., Uldall, P.R., The problem of renal preservation., Brit. J. Urol., 1966, 38: 653.

41. Kohno, H., Shiki, K., Yasutaka, U., Tokunaga, K., Cold storage of the rat heart for transplantation. J. Thorac. Cardiovasc. Surg., 1987, 93: 86.

42. Kolata, R.J., Standeven, J., Codd, J.E., Left ventricular function and high-energy phosphates after 24 hours of hypothermic preservation. J. Heart Transplant., 1984, 3(4): 319.

43. Kyosola, K., Harjula, A., Mattila, S., Aalto-Setalai, K., Mattila, I., Mattila, P., Salmengera, M., Merikallio, E., Cardioplegic protection with hypothermic K-Fluosol DA and K/Mg-Fluosol DA solutions. Scand. J. Thor. Cardiovasc. Surg., 1985, 19: 267.

44. Laks, H., Barner, H.B., Hahns, J.W., Standeven, J.W., Jellinek, M., Menz, L.J., Myocardial protection with intermittent coronary perfusion using cold blood versus a cardioplegic solution. Surg. Forum, 1977, 28: 231.

45. Lam, C.R., Geoghegan, T., Lepore, A., Induced cardiac arrest for intracardiac surgical procedures. J. Thorac. Surg., 1955, 30: 620.

46. Langendorf, O., Untersuchungen an Uberlebended Saugethierherzen. Pfluegers Arch., 1895, 61: 291.

47. Lazar, H.L., Foglia, R.P., Manganaro, A.J., Buckberg,

G.D., Detrimental effects of premature use of inotropic drugs to discontinue cardiopulmonary bypass. Surg. Forum, 1978, 29: 276.

48. Leaf, A., Cell swelling: A factor in ischemic tissue injury., Circulat., 1973, 48: 455.

49. Levitsky, S., Mullin, E.D., Sloane, R.E., Ferrans, V.J., Buja, M., Williams, W.H., Morrow, A.G., Effects of a hyperosmotic perfusate on extended preservation of the heart. Circulat., 1971, 43(Suppl.I): 124.

50. Levitsky, S., Williams, W.H., Detmer, D.E., McIntosh, C.L., Morrow, A.G., A functional evaluation of the preserved heart. J. Thorac. Cardiovasc. Surg., 1970, 60(5): 625.

51. Lindbergh, C.A., An apparatus for the culture of whole organs. J. Expt. Med., 1935, 62: 409.

52. Losman, J.G., Heart xenotransplantation in a newborn. J. Heart Transplant., 1984, 4: 10.

53. Lurie, K.G., Billingham, M.E., Masek, M.A., Ginsberg, R., Bristow, M.R., Reitz, B.A., Ultrastructural and functional studies on prolonged myocardial preservation in an experimental heart transplant model. J. Thorac. Cardiovasc. Surg., 1982, 84: 122.

54. Martin, D.R., Scott, D.F., Downes, G.L., Belzer, F.O., Primary cause of unsuccessful liver and heart preservation: cold sensitivity of the ATPase system. Ann. Surg., 1972, 175: 111.

55. Melrose, D.G., Dreyer, B., Bentall, H.H., Baker, J.B., Elective cardiac arrest: preliminary communication. The Lancet, 1955, 2: 21.

56. Mendez-Picon, G.J., Goldman, M.H., Wolfgang, T.C. Long-distance procurement and transportation of human hearts for transplantation. Heart Transplant., 1981, 1: 63.

57. Miller, L.W., Galbraith, T., Standeven, J., Kolata, R.J., Codd, J.E., Prolonged cardiac preservation. Surg. Forum, 1984, 35: 291.

58. Nakae, S., Webb, W.R., Salyer, K.F., Unal, M.O., Cook, W.A., Dodds, R.P., Williams, G.T. Extended survival of the normothermic anoxic heart with metabolic inhibitors. Ann. Thorac. Surg., 1967, 3(1): 37.

59. Noirclerc, M., Duvinage, J.F., Sudan, N., Tedeschi, J., Coutant, P., Lejeune, G., Mise au point d'un procede

simple de conservation du poumon assurant une reprise immediate de fonction apres vingt-quatre heures d'ischemie. Acta Chirurgica Belgica, 1972, No. 5.

60. Offerijns, F.G., Krijnen, H.W., The preservation of the rat heart in the frozen state. Cryobiol., 1972, 9: 289.

61. Opie, L.H., Effect of extracellular pH on function and metabolism of isolated perfused rat hearts. Am. J. Physiol. 1965, 209: 1075.

62. Proctor, E., Matthews, G. Archibald, J., Acute orthotopic transplantation of hearts stored for 72 hours, Thorax, 1971, 26: 99.

63. Proctor E., Parker, R., Preservation of isolated hearts for 72 hours. Br. Med. J., 1968, 4: 296.

64. Raithel, K.S., "20 years after first human heart transplant, 1987 may see 4000 procedures performed worldwide", J.A.M.A., 1987, 258(21): 3084.

65. Reitz, B.A., Brody, W.R., Hickey, P.R., Michaelis, L.L. Protection of the heart for 24 hours with intracellular (high K+) solution and hypothermia. Surg. Forum, 1974, 25: 149.

66. Sacks, S.A., Petritisch, P.H., Kaufman, J.J., Canine kidney preservation using a new perfusate. The Lancet, 1973, 1: 1024.

67. Schroeder, J.S., Hunt, S., Cardiac transplantation; update 1987. J.A.M.A., 1987, 258(21): 3142.

68. Sewell, D.H., Twomey, J.F., Neill, W.A., Nabseth, D.C., Cleveland, R.J., A protocol for ex vivo cardiac preservation, Surg. Forum, 1978, 29: 322.

69. Stewart, J.R., Gerhart, E.B., Wehr, C.J., Shuman, T., Merrill, W., Hammon, Jr., J.W., Bender, Jr., H.W. Free radical scavengers and myocardial preservation during transplantation. Ann Thorac. Surg., 1986, 42: 390.

70. Suzuki, S., Sasaki, H., Tomita, E., Matsuo, T., Sada, M., Mizuochi, I., Amemiya, H., Twenty-four hour preservation of canine hearts by retrograde coronary sinus perfusion. J. Heart Transplant., 1984, 4(1): 76.

71. Swanson, D.K., Dufek, J.H., Kahn, D.R., Improved myocardial preservation at four-degrees Celsius., Ann. Thorac. Surg., 1980, 30(6): 519.

72. Thomas, F.T., Schatzki, P.F., Hudson, B.H., Wolf, J.S., Successful 24-hour ischemic cardiac preservation using a new hyperosmolar perfusate. Surg. Forum, 1975, 26: 253.

73. Toledo-Pereyra, L.H., Hau, T., Simmons, R.L., Najarian, J.S., Lung preservation techniques. Ann. Thorac. Surg., 1977, 23: 487.

74. Trunkey, D., Degenhardt, T., Chartrand, C., Pryor, J., Belzer, F., Preservation of canine hearts. Cryobiol., 1970, 6(6): 515.

75. Tyers, G.F., Williams, E.H., Hughes, H.C., Todd, G.J., Effect of perfusate temperature on myocardial protection from ischemia. J. Thorac. Cardiovasc. Surg, 1977, 73(5): 766.

76. Tyers, G.F., Todd, G.J., Niebauer, I.M., Manley, N.J., Waldhausen, J.A., The mechanisim of myocardial damage following potassium citrate (Melrose) cardioplegia. Surgery, 1975, 78(1): 45.

77. Wallace, A.G., Skinner, Jr., N.S., Mitchell, J.H., Hemodynamic determinants of the maximal rate of rise of left ventricular pressure. Am. J. Physiol., 1963, 205: 30.

78. Watson, Jr., D.C., Consistent survival after prolonged donor heart preservation. Transplant. Proceed., 1977, 9(1): 297.

79. Watson, Jr., D.C., Gregg, D.L., Rhodes, G.R., Survival after orthotopic transplantation of hearts preserved for 48 hours, Surg. Forum, 1976, 27: 255.

80. Wicomb, W.N., Cooper, D.K., Novitzky, D., Barnard, C.N. Cardiac transplantation following storage of the donor heart by a portable hypothermic perfusion system. Ann. Thorac. Surg., 1984, 37(3): 243.

81. Wicomb, W.N., Cooper, D.K., Hassoulas, J., Orthotopic transplantation of the baboon heart after 24 hours preservation by continuous hypothermic perfusion with an oxygenated hyperosomolar solution. J. Thorac. Cardiovasc. Surg., 1982, 83: 133.

82. Windholz, M., editor. The Merck Index; an encylopedia of chemicals and drugs. 9th edition. Rahway, NJ: Merck, 1976.

83. -----, "Survival rates improve, age limits fall in

Stanford program.", Cardiol. World News, 1985, April: 15.

84. -----, Xenografts; review of the literature and current status (Council on Scientific Affairs Report), J.A.M.A. 1985, 254(23): 3353.

YALE MEDICAL LIBRARY

Manuscript Theses

Unpublished theses submitted for the Master's and Doctor's degrees and deposited in the Yale Medical Library are to be used only with due regard to the rights of the authors. Bibliographical references may be noted, but passages must not be copied without permission of the authors, and without proper credit being given in subsequent written or published work.

This thesis by has been used by the following persons, whose signatures attest their acceptance of the above restrictions.

NAME AND ADDRESS	DATE